Cold-Active Enzymes in Food Processing 19

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Abstract

Microorganisms living in extreme environmental conditions (extremophiles) are potential source of extremozymes; they possess utmost stability under extreme environmental conditions. Cold-active enzymes are extremozymes produced by the psychrophiles (extremophiles) and have attracted much attention as biocatalysts due to their capacity to resist unfavourable reaction conditions in the industrial process. Cold-active enzymes possess wide applications in the food industry; these enzymes are not only secreted by bacteria but also from yeasts and moulds. Although enzymes are derived from plant and animal sources, cold-active microbial enzymes have taken advantage, due to their productivity and thermostability. Psychrophilic microorganisms produce a wide range of cold-active enzymes with immune application in food processing. The use of ß-galactosidase for the removal of lactose from refrigerated milk, application of pectinase for the reduction of viscosity and turbidity in chilled juice and use of amylase for hydrolysis of polysaccharides in starch processing industries and processing of meat with the help of cold-active proteases are the representative examples of application of cold-active enzymes. Cold-active enzymes possess exceptional molecular flexibility that has opened up newer areas of applications. In food processing industries, cold-active pectinases have been used for the removal of pectin which is important in fruit juice and wine processing, coffee and tea processing and macerating of plants and vegetable tissue, for degumming of plant fibres, for extracting vegetable oils and for adding poultry feed and in the alcoholic beverages. To fulfil the demand of industries, enzyme technology needs extension of biotechnological approach in terms of both quality and quantity. The potential of cold-active enzymes provides numerous opportunities for industrial

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applications. However, specific properties of cold-active enzymes may be improved and modified through enzyme engineering.

Keywords
Extremophiles · Extremozymes · Psychrophiles · Cold-active enzymes · Food processing

19.1 Introduction

Microorganisms needing extreme environmental conditions for growth and development are known as extremophiles, and the enzymes they produce are called extremozymes. In 1974 MacElroy first time used the term extremophile. Microorganisms living in extreme environments include those with either high temperature (55–121 °C) or low temperature (−2 to 20 °C), high salinity (2–5 M NaCl) and high alkalinity (pH > 8) or high acidity (pH < 4) (Madigan and Marrs 1997; Rothschild and Manicinelli 2001). Extremophilic microorganisms are important source of extremozymes, which possess utmost stability under extreme environmental conditions. Therefore, plentiful attention has been given to the microorganisms that are able to thrive in extreme environments (extremophiles). It has been found that biocatalysis using extremophiles as well as extremozymes is swiftly being transformed from an academic science to an industrially viable technology to address the demands. Each group of the extremophiles possesses unique properties, which can be bound to provide enzymes with a wide range of application potentials (Adams et al. 1995; Hough and Danson 1999). Extremophiles are well adapted molecularly as well as structurally to withstand harsh environmental conditions. The biocatalysts known as extremozymes are proteins produced by microorganisms called extremophiles that function under extreme conditions. Because of their stability, extremozymes offer new opportunities for biocatalysis and biotransformation. The few examples of extremozymes produced by extremophiles are pectinases, proteases, keratinases, lipases, cellulases, amylases, xylanases, esterases, catalases, peroxidases and phytases. All these extremozymes have great potential for application in various biotechnological processes and in food processing.

19.2 Psychrophiles and Their Habitats

The name "psychrophile" is of Greek origin and the words "Psychros" mean cold and "Philos" mean loving (i.e. cold-loving). They have been called also "cryophiles" and "rhigophiles". Both of these words were derived also from Greek and also had the essential meaning as psychrophiles that was used first by Schmidt (1902). Microorganisms thriving at low temperatures are known for a long time (Morita 1966; Farrell and Rose 1967). Psychrophilic organisms have been classified in two groups (Stokes 1963):

1. Obligate psychrophiles are having optimal growth temperature of ≤20 °C.
2. Facultative psychrophiles are having optimal growth temperature of >20 °C.

Cold-adapted microorganisms are broadly categorized into psychrophiles as they are cold-loving microorganisms and they show optimal growth at less than 15 °C and psychrotrophs as they are cold-tolerant microorganisms and they show optimal growth at 20–25 °C. (Helmke and Weyland 2004; Cowan et al. 2007). The notable features of cold-adapted microorganisms are their survival at low temperatures, due to increased unsaturated fatty acid content in the membrane and increased solute concentrations inside the cells (D'Amico et al. 2006; De Maayer et al. 2014). Microorganisms and other higher organisms inhabit places like cold water, ice, frozen soil, glaciers, etc. Microorganisms inhabiting at these low temperatures function well and are often found in combination with other extreme environmental conditions, for example, high salinity, low pH, high pH and high pressure. Mykytczuk et al. (2013) reported the bacteria *Planococcus halocryophilus* Or1 that were isolated from high Arctic permafrost showed virtuous growth and reproduce at −15 °C. It has been reported that metabolically active bacteria inhabiting at extreme low temperatures as low as −32 °C (Bakermans and Skidmore 2011) show possibility of life even further.

Both categories of extremophilic microorganisms like psychrophilic (cold-loving) or psychrotolerant (cold-adapted) are found residing at low temperatures of the earth, which include high mountains, glaciers, ocean depths, polar areas, shallow subterranean and refrigeration appliances. They are also found on the surfaces of plants and animals living in cold environments, where temperatures never exceed 5 °C. The mesophilic yeasts grow between 5 °C and 35 °C. An extensive variety of cold-loving microorganisms have been found inhabiting these low-temperature environments as some of them belong to gram-negative bacteria, e.g. *Pseudoalteromonas* sp., *Moraxella* sp., *Psychrobacter* sp., *Polaromonas* sp., *Psychroflexus* sp., *Polaribacter* sp., *Moritella* sp., *Vibrio* sp. and *Pseudomonas* sp.; some belong to gram-positive bacteria, e.g. *Arthrobacter* sp., *Bacillus* sp. and *Micrococcus* sp.; some archaea also belong to psychrophiles, e.g. *Methanogenium* sp., *Methanococcoides* sp. and *Halorubrum* sp.; yeasts like *Candida* sp. and *Cryptococcus* sp.; and fungi like *Penicillium* sp. and *Cladosporium* sp. have also been isolated from cold environments (Cavicchioli et al. 2002; Deming 2002; Margesin et al. 2002; Feller and Gerday 2003; Georlette et al. 2004). Permanently cold environments such as the polar region, marine environment and deep water are the places where psychrophiles or cold-loving microorganisms are mostly found (Sabri et al. 2001; D'Amico et al. 2006). Environments where psychrophilic and psychrotrophic microorganisms are inhabiting are supposed to work on the biodegradation of organic matter and the cycling of essential nutrients (Lambo and Patel 2006; Welander 2005; Ruberto et al. 2005). It has been reported that cold-adapted microorganisms provide an extensive biotechnological prospective over the use of organisms and their enzymes which operate at higher temperatures (Georlette et al. 2004; Margesin et al. 2002). There are quite a lot of potential applications of cold-adapted enzymes in the food processing industries

(Margesin and Schinner 1994). Psychrophilic microorganisms are required to swell the important applications of cold-active enzymes in different areas of food processing.

19.3 Cold-Active Enzymes

Enzymes used in different industries have developed into a multibillion-dollar worldwide market due to their versatile applications. It is estimated that worldwide market for industrial enzymes will reach $7 billion by 2018 with a composite annual growth rate of 8.2% from 2013 to 2018 (Dewan 2014). The enzymes used in food industries are mentioned in Table 19.1. Psychrophilic microorganisms are beneficial sources of cold-active enzymes as these microorganisms are skilled of growing in extremely low-temperature environments, such as deep seas and glaciers in the polar regions. Cold-active enzymes (e.g. pectinase, proteases, amylases, lipases and cellulases), unique cold-shock and cold accumulation proteins of psychrophilic microorganisms, are having a wide range of biotechnological applications (Gounot 1991). Enzymes are known as protein catalysts as they are synthesized by living systems and possess importance in different synthetic as well as degradative process. The applications of psychrophilic or cold-adapted enzymes in the food industry are wide. The most representative example of cold-active enzymes is the use of β-galactosidase enzyme for removal of lactose from refrigerated milk, and one more example is application of pectinase for reduction of viscosity and turbidity in chilled juice. Enzymes from organisms which are adapted to cold environment will retain their activities at low temperature. Studies on psychrophilic microorganisms that inhabit cold environment are carried out for the economic value of their cold-

Table 19.1 Enzymes used in food industries

Dairy production	Brewing	Baking	Wine and fruit juice	Meat
Rennet	β-Glucanase	Maltogenic amylase	Pectinase	Protease
Lactase	α-Amylase	Glucose oxidase	β-Glucanase	Papain
Protease	Protease	Pentosenase		
Catalases	Amylogluco-sidase			
				

active properties for novel industrial applications. In food processing industries to avoid spoilage of foods and changes in taste and nutritional value of foodstuffs they are treated under mild conditions at an ambient temperature. Hence, different cold-active enzymes are used for processing foods for maintaining quality of food products, (Margesin and Schinner 1994; Russell and Hamamoto 1998; Gerday et al. 2000). As psychrophilic microorganisms are showing optimal growth at 15 °C (Morita 1975), this reason has attracted the attention of scientists to use them as sources of cold-active enzymes with potential for low-temperature catalysis. In various domestic processes cold-active enzymes can also be beneficial. It has been reported that psychrophilic yeast and their cold-active enzymes possess potential biotechnological uses in different industries which include food, pharmaceutical, textile, beverage and detergent industries Hamid et al. (2014). Table 19.1 shows enzymes used in food industries.

Cold-active enzymes have the ability to catalyse different reactions at low temperatures which offers great industrial and biotechnological potential (Van den Burg 2003). These cold-active enzymes from psychrophilic microorganisms can be used in food processing industries, for clarification of fruit juice and other processes executed at low temperature (Table 19.2). Psychrophilic microorganisms produce cold-active enzymes like amylases, proteases, lipases, cellulases, pectinases, β-glucosidases, β-galactosidases, peroxidases, chitinases, lysozyme, etc. – these enzymes could be isolated and characterized as they possess vast industrial applications. These cold-active enzymes have high specific activities at lower temperatures (Feller and Gerday 2003), and not only this but these enzymes also have the capability to support transcription and translation at low temperatures (Goodchild et al. 2004). Cold-active enzymes own high activities at low and moderate temperatures; thus they offer potential economic profits (Cavicchioli and Thomas 2000). To avoid or prevent foodstuff from microbial spoilage and also to avoid change in nutritional contents and flavour, food is treated with low temperatures. The time span of cold storage of food products can be used for additional fermentation and optimization, if microorganisms or their enzymes are added that operate in the low-temperature range. Different scientists have reviewed the capabilities or potential of psychrophiles and their cold-active enzymes along with their application in different industries (Cavicchioli et al. (2002), Deming (2002), Margesin et al. (2002), Feller and Gerday (2003) and Georlette et al. (2004)). The prospective of cold-active enzymes in maintenance of lowering of temperature without loss of efficiency and carrying out processes, which results in saving of energy consumption, has a great potential for numerous biotechnological processes (Kuddus and Ramteke 2008). Due to significance of these special features, the reaction rate of psychrophilic enzymes decrease more slowly as compared to enzymes from mesophilic or thermophilic microorganisms when the temperature decreases (Feller 2013). In a comparative study of cold-adapted and mesophilic xylanases, conducted by Collins and collaborators, it has been observed that cold-adapted enzymes are more vigorous at low temperatures but more thermolabile as the temperature increases (Collins et al. 2002). Table 19.2 shows application of cold-active enzymes in food processing.

Table 19.2 Application of cold-active enzymes in food industry

Enzymes	Application	References
Lipase, protease, phytase, glucanases, xylanase	These cold-active microbial enzymes are used in animal feeds for the improvement of digestibility and assimilation. They carry various applications in food processing companies	Hatti-Kaul et al. (2006), Tutino et al. (2009), Ueda et al. (2010), Collins et al. (2005)
Protease	For the removal of hemicellulosic material from feed, proteases are highly used	Wang et al. (2010)
Chitinase	Chitinases are having vast applications in tenderization of meat	Dahiya et al. (2006)
α-amylase,	Single-cell protein from shellfish waste	Gerday et al. (2000)
Pectinase, xylanase	Mostly hydrolysis of starch is carried out by application of these enzymes	Nakagawa et al. (2004), Collins et al. (2005)
Pectate lyase, pectinase	For easy extraction and clarification of fruit juices, vegetable juices and wine processing pectinases and pectate, lyase play a vital role	Truong et al. (2001)
α-Amylase, xylanase	In dairy industry for ripening of cheese, these microbial enzymes are used	Gerday et al. (2000), Collins et al. (2005)
β-galactosidase	Lactases possess wide application in dough fermentation and in other bakery products	Białkowska et al. (2009)
Laccase	Removal of lactose from milk, conversion of lactose in whey into glucose and galactose in dairy industry	Kunamneni et al. (2008)
Feruloyl esterase	In beverage industries this enzyme is used for wine and beverage stabilization. It is also used for production of vanillin as a food precursor	Aurilia et al. (2008)

19.4 Cold-Active Enzymes and Their Applications in Food Processing

It is not new but from long back microbial enzymes are used in foods and beverages. But in current food processing and beverage industries, the drift is to switch high-temperature processes with low-temperature processes as it will help in maintaining the quality of the product. It has been found that low-temperature processing delivers commercial, economic and environmental benefits. The most fruitful advantages of low-temperature food processing are prevention of products from food spoilage and contamination, energy savings, the foodstuff will retain labile and volatile flavour compounds, quality of taste will be maintained and most importantly undesirable chemical reactions that may occur at higher temperatures will be minimized. Along these benefits, there will be higher control over cold-active enzymes as they can be inactivated at high temperatures (Pulicherla et al. 2011; Horikoshi 1999). Low-temperature processing will help in controlling the quality of material or foodstuff. The most focused application of enzymes in the baking industry is improvement of quality of bread including taste, and in the beverage industry, these potential enzymes are used in maintaining wine colour and clarity, and they are also used for reducing sulphur content. To boost the filterability and to develop or improve the flavour of final products, different industrial enzymes can be used. In regard to food and beverage enzymes, they make up the largest market for industrial enzyme applications. Some important microbial enzymes like α-amylases, peptide hydrolases, lipases, pectinase, lactase and catalases are added to foodstuff during food processing to improve specific characteristics of the food. Cold-active enzymes are also used for environmental bioremediation processes which include digesters, composting and oil degradation or xenobiotic biology applications; they are also applicable in molecular biology, biotransformation and heterologous gene expression in psychrophilic hosts to prevent formation of inclusion bodies (Feller et al. 1996).

19.4.1 Amylases

Group of enzymes called amylases have been found in different microorganisms like bacteria (Haseltine et al. 1996), fungi (Fadel 2000). Amylases possess vast and important industrial application as it is one of the most important industrial enzymes. As to the cold-active amylases, hardly any class of microbes other than bacteria of Antarctica origin has been explored (D'Amico et al. 2003). Therefore, cold-active amylases from such bacteria have become the model for biochemical study (D'Amico et al. 2003) and contemplating future applications. As the demand for enzymes is increasing in various industries and biotechnological sector, there is a huge attentiveness in developing enzymes along with the improved properties. As we have an example of raw starch degrading amylases appropriate for industrial applications and their cost-effective production techniques (Burhan et al. 2003). Though amylases originate from different sources like plants, animals and microorganisms, the amylases from microbial origin are the maximum produced due to their productivity and thermostability and are highly used in industries (Burhan et al. 2003). They are an important group of enzymes that are employed in food, textiles, paper and fermentation industries. In biotechnology, amylases are among the most important enzymes used, especially in the process of starch hydrolysis. It is because of high catalytic activities at low temperature, low thermostability and unusual specificities of cold-active enzyme which offer innovative opportunities for biotechnological exploitation (Russell 2000). It has been found that industrial coconut milk waste could be used as substrate for the production of α-amylase and it would be a good way to reduce importation of the enzymes from other countries

(Haki and Rakshit 2003). *Alteromonas haloplanktis*, an Antarctic bacterium, was the first cold-adapted α-amylase which was extensively studied and has been magnificently expressed in the mesophilic host *E. coli* (Feller et al. 1998). Some other cold-active α-amylases, like extracellular α-amylase from *Microbacterium foliorum* GA2, was isolated from the Gangotri Glacier (Kuddus et al. 2012) and other cold-active α-amylase from marine bacterium *Z. profunda* (Qin et al. 2014); they signify as worthy candidates for application in this particular industry. Yeast isolates *Rhodotorula mucilaginosa* PT1 and *Cystofilobasidium capitatum* SPY11 producing cold-active α-amylase with potential applications in food, textile and detergent industries have been reported (Hamid 2016). With the emergence of new boundaries in biotechnology, the scope of amylases has extended beyond their conventional applications in starch saccharification, textile, food, brewing and distilling industries to several other fields, which include medicinal, clinical and analytical chemistry (Pandey et al. 2000). Other than this crystallographic structures of various cold-adapted microbial enzymes have been elucidated α-amylase (Aghajari et al. 1998). It has been reported by Borchert et al. (2004) in a patent relating to a variant of the parent α-amylase from *Bacillus licheniform*is. This was displaying increased specific activity at temperatures from 10 to 60 °C and was granted to Novozymes in 2004.

19.4.2 Lactases

In our daily intake, carbohydrate lactose is the main part. Lactose is hydrolysed by β-galactosidase into glucose and galactose; it is commonly known as lactase (Shukla and Wierzbicki 1975). Lactose is abundantly found in milk, but it cannot be directly taken up by humans. Intolerance of lactose is severely affecting a large portion of the people as estimated up to 50 million in the USA. To get rid of this intolerance problem, economical source of β-galactosidase for effective production of lactose-hydrolysed dairy products has a significant potential (Bury et al. 2001) as the lactose possesses low relative sweetness and solubility. It has been found that disproportionate lactose in the large intestine can cause tissue dehydration due to osmotic effects and poor calcium absorption due to low acidity. Furthermore, fermentation of lactose by intestinal microflora results in fermentative diarrhoea, bloating, flatulence, blanching and cramps and watery diarrhoea (Shukla and Wierzbicki 1975). Lactose is a hygroscopic sugar; it carries high affinity to captivate flavours and odours. Due to this reason, it leads to different imperfections or problems in refrigerated foods; few defects we are mentioning here are crystallization in dairy foods, growth of sandy texture and deposit formation (Carrara and Rubiolo 1994). Potential microorganisms producing β-galactosidase had been broadly studied due to their valuable applications. Most famous application of food enzymes in dairy industry is that they are used for cheese production and also in the preparation of various dairy products. To hydrolyse lactose in milk, β-galactosidase from *Kluyveromyces lactis* is used for the manufacturing of lactose-free products (Mateo et al. 2004).

The β-galactosidase has potential applications in food processing industry. It is usually known as lactase and is an important food enzyme. β-galactosidase or lactase hydrolyses lactose into glucose and galactose. A large portion of the population indicates lactose intolerance; it's because of low levels of enzyme in the intestine that can't act on lactose and leads to this problem. Most people suffering from lactose intolerance have difficulty in consuming milk and dairy products. Cold-adapted β-galactosidase is a significant food industrial enzyme, because it is used for removal of lactose from milk at refrigerated conditions or low temperature so that it can be digested and can be consumed by the lactose-intolerant portion of population. Cold-adapted β-galactosidase is also used for conversion of lactose into whey, which is a by-product of the cheese industry, from a pollutant to more readily fermentable glucose and galactose. Large amounts of whey are generated as a by-product in the cheese manufacturing industry; it's actually the aqueous fraction of milk which is produced during cheese processing. About 3–8% of lactose is present in the whey, and the main solute in cheese whey is lactose (Speer 1998). For the reduction of lactose content in milk, β-galactosidase produced by *K. lactis* has been used for its industrial potential (Suarez et al. 1995). It has been observed that lactose gets easily crystallized; this reason creates limits and boundaries for its applications to few processes in the dairy industries. Cheese that is manufactured from hydrolysed milk shows ripening quickly than that made from normal milk. Moreover, the hydrolysis of whey converts lactose into different beneficial product (e.g. sweet syrup). These useful products can be used in different processes carried out in dairy, confectionary or baking and soft drink industries (Tweedie et al. 1978; Pivarnik et al. 1995). Various significant genes coding for cold-active β-galactosidase have been identified in different microorganisms including *Arthrobacter* (Coker et al. 2003; Nakagawa et al. 2003), *Pseudoalteromonas* (Hoyoux et al. 2001; Fernandez et al. 2002) and *Rahnella aquatilis* (Park et al. 2006); these genes were located in yeasts too (Nakagawa et al. 2006a, b). Comparative study of different commercially existing food-grade cold-active enzyme β-galactosidases has been carried out, and it was demonstrated that these enzymes are satisfactorily working in milk at low temperatures to enable hydrolysis of lactose (Horner et al. 2011).

To reduce the cost of manufacturing lactose-free products, potential cold-active β-galactosidases can be used. Marine psychrophilic bacterium producing cold-active β-galactosidase has been characterized recently. β-Galactosidases secreted by marine psychrophilic bacterium hydrolysed around 80% of lactose in raw milk at 20 °C and pH 6.5; this property implies its potentiality in dairy industry (Ghosh et al. 2012; Pulicherla et al. 2013). The work on removal of lactose from milk by psychrophilic β-galactosidase during refrigeration conditions has recently been patented. As per industrial applications, yeasts are known as the best and essential source of β-galactosidase. Yeasts are found to be suitable for hydrolysis of lactose in milk due to their neutral pH optima; they are gaining attention widely and are considered safe for use in foods. On the production of β-galactosidase from diverse yeast strains, a lot of research has been carried out for their potential applications in different industries. Hamid et al. 2013 report two yeasts secreting cold-active β-galactosidases that are capable of hydrolysing lactose at lower temperatures, reflecting novel application in dairy industry. Application of lactase for reduction of lactose percentage in different dairy products seems a suitable procedure to increase their uses and to tackle with the complications of lactose insolubility. Treatment of lactase can make milk, more fit food, accessible to large portion of population including adults as well as children that are lactose intolerant.

19.4.3 Pectinases

Pectinases are enzymes which break down polysaccharide pectin of plants into simpler molecules, i.e. galacturonic acids. From long back pectinases are used to increase clarity and yield of fruit juices. Pectinases are not found in specific group of organisms, but they are most widely distributed enzymes in bacteria, fungi and plants. These enzymes are very essential part of the food processing industries. Pectin is found in primary cell walls and the middle lamellae of higher plant cells. It is an important constituent of plants along with cellulose and xyloglucan. Pectin is hetero polysaccharide, mostly composed of D-galacturonic acid residues connected with α-1, 4-linkages that form homogalacturonan chains, and the D-galacturonic acid residues may be methylated (Sakai et al. 1993). During various stages of juice processing in food industries like extraction, filtration, concentration and clarification of fruit juice, pectin compound causes difficulties in all processes (Pilnik and Rombouts 1985). Wide range of microorganisms such as bacteria, fungi, yeast and actinomycetes produced pectinases; along these microorganisms some plant pathogenic fungi and bacteria also secrete pectinases. Pectinases which break down pectin polysaccharides are classified as per their substrate preference, pattern of action on the galacturonan backbone of the polymers and most importantly mechanism of reaction (Collmer et al. 1988; Bonnin 2003). Nakagawa et al. 2002 reported psychrophilic yeast *Cystofilobasidium capitatum* PPY-1 which produces cold-active pectinolytic enzymes having wide applications in food industry. To commercialize microbial pectinases, it's important to enriched knowledge about their properties, so that it becomes possible to go for industrial production and to apply these enzymes in several possible fields (Gummadi and Panda 2003). Cold-active pectinolytic enzymes produced by *Rhodotorula mucilaginosa* PT1 and *Cystofilobasidium capitatum* SPY11 have maximal activity at low temperatures, and this property of enzyme makes it appropriate for wine production and juice clarification (Sahay et al. 2013). Pectin, found in plants, is being degraded by enzyme pectinase; these enzymes possess several food-associated applications and are highly used in this sector; they are used in fruit juice processing for clarification and viscosity reductions and vinification and extraction of natural oils (Adapa et al. 2014). Pectinases possess immune biotechnological application; far-reaching research has been carried out in respect of isolation, characterization and production of the pectinase from microorganisms. These enzymes are known as the most essential groups of industrial enzymes having application in different industries like pectinase which are employed in fruit and vegetable industry; they are applied in improvement of cloud stability of fruit nectars and used for clarification of fruit juices and wines and they are also used in coffee and tea processing, softening of vegetable tissue and papermaking (Soares 2001; Reid and Ricard 2002). Pectinases are used in various industrial processes where removal of pectin is necessary; the following are few important processes where pectinase are employed: applied in coffee and tea processing, softening of plants and vegetable tissue, fruit juice processing, degumming of plant fibres, waste water treatment, removing vegetable oil, bleaching of paper and adding poultry feed and in the textile, alcoholic beverages and food industries. Therefore, pectinases are widely employed for the degradation of pectin in the fruit and vegetable industries (Alkorta et al. 1998). It is estimated that about 0.5–4% pectin compound is found in weight of fresh material. Once the raw material is pressed, the main content of raw juice is rich in insoluble particles of pectic substances. In complete grounded state, the pectin is found in the liquid phase, i.e. soluble pectin, which leads to an increase in viscosity and the pulp particles. Extracting juice by pressing or by following any other mechanical procedure is very difficult. The valuable tools for extraction of juices are pectinases. Pectinases help in reduction of viscosity of the fruit juice, and the press ability of the pulp increases; they are used for easy extraction along high yield of juices, and the jelly structure disintegrates. They are produced during the natural ripening process of same fruits. Pectinases are employed in textile industries for treatment of natural fibres, like linen and ramie fibres (Baracat et al. 1991).

19.4.4 Lipases

For the protein polymerization and gelling in fish flesh, lipases are being used from long ago (Cavicchioli and Siddiqui 2004). It has been found that cold-adapted lipases are mostly distributed in microorganisms which are habitant of low-temperature areas nearly 5 °C. There are a wide range of sources which produce lipases, but only few bacteria and yeast were explored for the production of cold-adapted lipases (Joseph 2006); most other sources are unexplored. Researchers are continuously working on isolation and characterization of cold-active lipases from these psychrophilic microorganisms which are having high activity at low temperatures. Cold-active lipases are employed for interesterification of fats and production of fatty acids (Jaeger and Eggert 2002). Lipases secreted by psychrophilic microorganisms possess vast application in food industries. In modern food processing industries, lipases are used as key enzyme, and it has become a vital part of current food industries from different aspects. In the past decade, focus has been given to the enzymes for improvement of traditional chemical processes of food manufacturing. Although microbial lipases are very important components for food processing, few microorganisms including psychrotrophic bacteria of *Pseudomonas* sp. and a few moulds of *Rhizopus* sp. and *Mucor* sp. caused chaos with milk and dairy products and soft fruits. Cold-active lipase from *Pseudomonas* strain P38 is widely used in nonaqueous biotransformation for the synthesis of n-heptane of the flavouring compound butyl caprylate (Tan et al. 1996). Cold-active lipases have been used in improvement of food texture and flavour modification (Cavicchioli and Siddiqui 2004). Other than these applications, immobilized lipases from *C. antarctica* (CAL-B), *C. cylindracea* AY30, *H. lanuginosa*, *Pseudomonas* sp.

and *Geotrichum candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants in sunflower oil (Buisman et al. (1998)). For storage and preservation of foodstuff, refrigeration technology is widely used, but due to high nutrient contents in foodstuff, a great diversity of some common psychrotolerant food spoilage microorganisms is found. Cold-active lipases were isolated from refrigerated milk and food samples from the bacterial genera including *Pseudomonas fragi* (Aoyama et al. 1988; Alquati et al. 2002), *Pseudomonas fluorescens* (Dieckelmann et al. 1998) and *Serratia marcescens* (Abdou 2003). According to Collins et al. (2002), lipases form psychrophilic microorganisms which can be virtuous substitute to mesophilic enzymes as they can be used in different industries like brewing industry and wine industries, animal feed supplements and cheese manufacturing.

19.4.5 Proteases

To overcome the problem of food spoilage and to maintain taste and nutritional value of foodstuffs, industries are treating foods with ambient temperatures, and for this reason cold-active enzymes are used for processing foods (Margesin and Schinner 1994; Russell and Hamamoto 1998; Gerday et al. 2000). For clarification of fruit juices, maximum studies are carried on screening of yeasts for potential enzyme production having target of proteinase or pectinase, because they are essential enzymes used by juice industry (Braga et al. 1998; Trindade et al. 2002). Cold-active proteases have abundant use in food industry, and wide range of microorganisms thriving in low temperatures secretes cold-active proteases used in different industries. Cold-active proteases from psychrophilic microbes are used in food industry; the most important application of cold-active proteases is softening and taste development of refrigerated meat products. For the rapid inactivation of cold-active proteases, mild heat treatment can result in inactivation of enzymes showing nature of thermal liability (Margesin et al. 2002). This property of cold-active proteases will be advantageous in preserving the quality of foodstuff in food industry. It has been found that alkaline proteases are used for the preparation of highly nutritional protein hydrolysates. Protein hydrolysates possess multiple applications in different areas as it plays a key role in regulation of blood pressure and it is used in infant food formulations. Other than this it is employed in specific therapeutic dietary products and for fortification of fruit juices and soft drinks (Neklyudov et al. 2000; Ward 1985).

19.4.6 Xylanases

Xylanases have shown potential applications in bread making in bakery industry. Three cold-active xylanases from *P. haloplanktis* TAH3A, *Flavobacterium* sp. MSY-2 and one from an unknown bacterial source efficiently enhanced dough properties along final bread volume up to 28%. These results were obtained when cold-active xylanases was compared with mesophilic xylanases from *Bacillus subtilis* and *Aspergillus aculeatus* (Dornez et al. 2011). Studies are being conducted from last few years on numerous novel cold-adapted xylanases isolated from different microorganisms (Wang et al. 2012; Chen et al. 2013; Del-Cid et al. 2014). Xylanases are used for the conversion of insoluble hemicellulose of dough into soluble sugars in bread making, and it happens before the baking process. It produces cottony but strong dough and increases size of loaves; it also makes it soft and increases elastic properties. All these essential properties of bread are obtained at low temperatures before baking of bread, so here the application of cold-active xylanases may provide a significant benefit and can be used for various processes in bakery industry. Even though they possess prospective applications, they have been rarely studied, and it has been found that currently a number of xylanases used in industries appear to be mesophilic or thermophilic in nature (Collins et al. 2005).

19.5 Conclusion

It's expected that more cold-active and cold-adapted microbial enzymes will be discovered in the near future, due to their potentiality and characteristics that are highly needed in industries. Cold-active enzymes from psychrophilic microorganism can be most beneficial in food industries as they possess various advantages over the enzymes from other sources. The cold-active enzymes and their psychrophilic microbial sources cover a wide range of industrial applications. Cold-active enzymes from different sources are being used as additives in detergents used for cold washing and as additives in food industries in fermentation, cheese manufacture, bakery and meat tenderizing. Cold-active enzymes from microbial origin are having interesting biotechnological as well as industrial applications. The few potential applications of these enzymes are mentioned here; some of them (e.g. β-galactosidase) are used for hydrolysis of lactose in milk, application of cellulases for bio-polishing and stone washing of textile products; pectinases are employed for extraction and clarification of fruit juices in food industry and for tenderization of meat and for improvement of taste in refrigerated meat using proteases. There is tremendous potential and use of microbial cold-active enzymes in different food industries. Due to their high stability at low temperatures, they are attracting more attention and are needed to be explored.

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